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HIGH DENSITY CULTURING OF THE BRINE SHRIMP, *Artemia salina* L.

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Simple to construct and inexpensive high density culturing techniques are described for culturing 2000 *Artemia* nauplii in 1-l bottles; 25 000 in 10-l bottles and 50 000 in 30-l plastic sheet cylinders.

Frozen *Dunaliella* or dried *Scenedesmus* cells are used as food. Growth results of these high density cultures are comparable with the best growth data reported in literature. The latter however refer to low-density experiments.

INTRODUCTION

Culturing *Artemia* larvae in high densities either as food for larval fishes or for fundamental investigation on radiation, toxicity or accumulation of pollutants, has until now embarassed the scientists faced with this problem.

As we soon concluded, the *Artemia* culturing systems, described in the literature, are not suited for high density rearing (Bowen, 1962; Reeve, 1963; Walne, 1967).

Even the culturing apparatus which we have recently developed is mainly for fundamental small density investigations (Sorgeloos and Persoone, 1972; Sorgeloos, 1973a).

This paper describes some methods to culture densities of approximately 2 000 larvae in 1-l bottles; 25 000 in 10-l bottles and 50 000 in 30-l plastic sheet cyclinders, i.e. concentrations from 1 to 2 larvae per ml.

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METHODS

All culture experiments are conducted in a conditionned culture room at 28 ± 0.5 °C (Teramoto and Kinoshita, 1961). As artificial sea-water medium we used the salt-mixture of Dietrich and Kalle (1963). Experiments in progress however indicate that even a less complex medium can fulfill the needs of these crustaceans.

Dry cysts are hatched in the culture vessel I (cf. Sorgeloos and Persoone, 1972). The nauplii are isolated from the hatching debris with the separator box (cf. Persoone and Sorgeloos, 1972) and counted with the apparatus described by Van Outryve and Sorgeloos (1973).

According to the quantitative needs, the larvae are cultured in glass

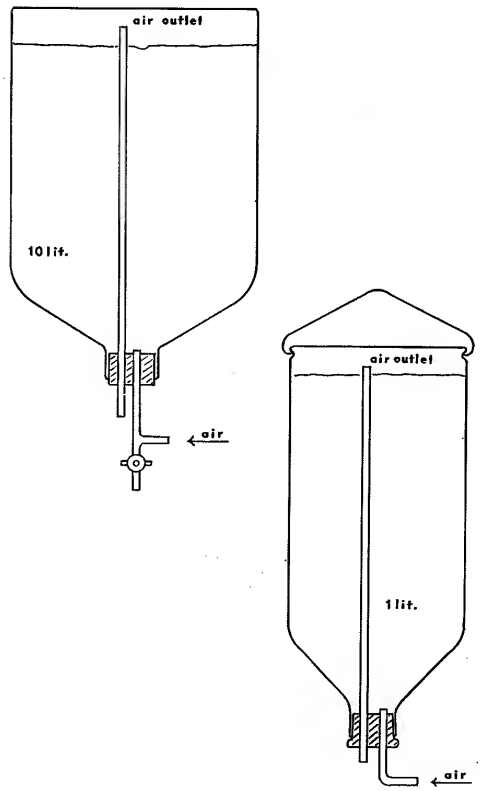

Fig. 1. Sketches of 1-l and 10-l culturing bottles.

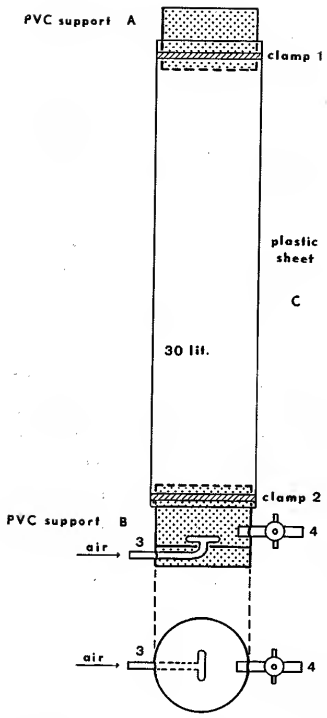

Fig. 2. Sketch of 30-l plastic sheet culturing cylinder.

bottles of 1- or 10-l content (Fig. 1), or in plastic sheet cylinders (Fig. 2) with an internal volume of maximum 30 l.

Both the 1-l bottles and the 10-l flasks are used in an upside down position, the former (serum bottles) being very conveniently hung up by their metal clamp whereas the latter are mounted in frames.

The rubber stoppers of both types are provided with an inlet and outlet tube.

The 30-l cylinder is made of a long (2 meter) very thin (0.2 mm) transparent polyethylene cylinder of 16 cm diameter; the extremities of which are clamped to two PVC cylinders of the same diameter (thickness of 1 cm).

The culturing cylinder is mounted vertically into a metal frame. PVC cylinder A being open while B has a waterproof bottom; holes for an air inlet (a T-piece with surplus pores) and for a drain (4) with stop-cock are provided.

As we already mentioned elsewhere (Sorgeloos, 1973a) *Artemia* larvae should at best be cultured with aeration by air-bubbling during one minute every half hour. The air is supplied by an air-pump which is automatically switched on for one minute, every half hour, by an electric clock (Micro Flash, type SYA 8021, "La Vedette", 67 Saverne, France)*.

In the 1- and 10-l bottles, the larvae are fed twice daily with about 50 000 frozen *Dunaliella* cells per individual (Sorgeloos, 1973a) from day 1 to 4; and 100 000 cells on days 5 to 8.

In another paper (Sorgeloos, 1973b) we proved that broken *Scenedesmus* cells constitute a good food for brine shrimps. Since those algae are already cultured in mass-culture pilot-plants (Soeder *et al.*, 1971) we used dried *Scenedesmus* powder (Mikrozell, Dohse Aquaristik, Bonn, Germany)* as food to culture the *Artemia* in the 30-l cylinders.

The 50 000 larvae are also fed twice daily; on the first 3 days they receive 2 g Mikrozell; on days 3 and 4, 2.5 g, and from day 5 on, they are fed each morning and evening a Mikrozell-quantity exceeding the ratio of the previous day by 0.5 g. Every three days the culture media are renewed; the contents of the culture flask or cylinder are poured on a screen (with suitable meshes) and the larvae are resuspended in fresh seawater.

Since we discovered that the *Artemia* larvae grow faster in darkness than under continuous illumination (Sorgeloos, 1973c), we culture them in complete darkness.

At the moment of renewing the medium, 30 larvae from each vessel are sampled, put in small PVC vials and frozen at −16 °C.

To determine the growth rate of the culture, the larvae are thawed and individually transferred to glass slides. As growth criterion we used total length as preconized among others by Gilchrist (1960). Using a projection system, mounted on a microscope, the total length from the anterior tip of the head in front of the ocellus, to the base of the caudal furca was measured with a curve meter. The mean length in microns and the standard deviation of the mean were computed.

RESULTS AND DISCUSSION

During the experiments and especially at the moments of changing the medium, we always found that mortality was very low. Growth data (mean total length in microns and standard deviation of the mean) are summarized in Tables 1, 2 and 3; larval growth curves are represented in Fig. 3.

*Use of trade names does not imply endorsement of commercial products.

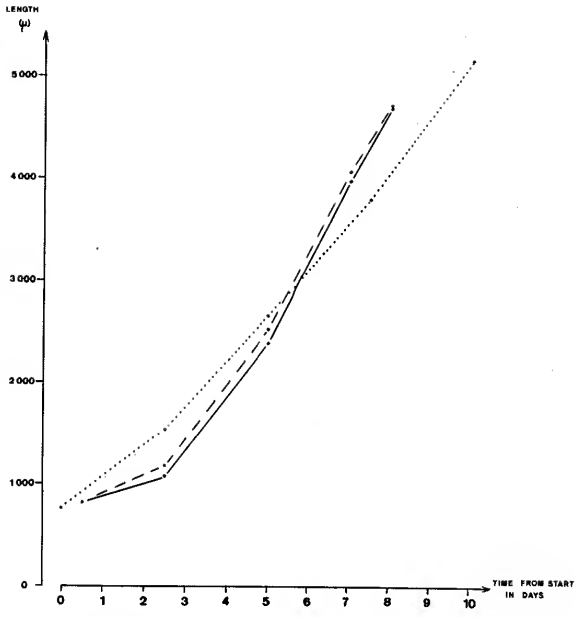

Fig. 3. Larval growth curve in the 1-l (——), 10-l (– – –) and 30-l (—·—) culture experiments.

TABLE 1

Growth data; 1-l culture bottle

Mean total length (μ) and standard deviation of the mean of *Artemia* larvae.

Time after starting experiments (hours)	Mean length (μ)	Standard deviation of the mean (μ)
0	818	63
48	1,082	68
110	2,390	291
158	3,984	618
182	4,702	690

From these results it is clear that the larvae show a rather comparable growth rate in the three types of culture vessels. The present results on brine shrimp culturing with these techniques are comparable with the best growth data reported in literature (Teramoto and Kinoshita, 1961; Walne, 1967; Von Hentig, 1971; Sorgeloos, 1973b). However the experiments of

TABLE 2

Growth data; 10-l culture bottle.

Time after starting experiments (hours)	Mean length (μ)	Standard deviation of the mean (μ)
0	818	63
48	1,180	72
110	2,534	262
158	4,073	526
182	4,726	491

TABLE 3

Growth data; 30-l culture cylinder

Time after starting experiments (hours)	Mean length (μ)	Standard deviation of the mean (μ)
0	770	83
58	1,540	175
120	2,664	393
178	3.790	721
240	5,165	780

these authors were only conducted with low larval densities (1 individual per 10 to 100 ml medium) in comparison with the larval densities of 1 to 2.5 individuals per ml in our mass-culture experiments.

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